

CLAIMS

- 10039890-010402
1. A method for obtaining a DNA complementary to a mRNA, the method comprising:
contacting the mRNA having a polyadenosine (polyA) tail with a primer mixture, the mixture comprising a plurality of primers wherein each primer comprises at least 5 contiguous deoxythymidines and at least 2 independently selected non-deoxythymidine nucleotides near one end; and
reverse transcribing the mRNA using a reverse transcriptase to produce a DNA strand complementary to the mRNA.
 2. The method of claim 1, wherein each primer further comprises a restriction enzyme sequence near the end opposite to the one containing the non-deoxythymidine nucleotides.
 3. The method of claim 2, wherein the restriction enzyme sequence is double stranded.
 4. The method of claim 1, wherein each primer comprises at least 10 contiguous deoxythymidines.
 5. The method of claim 1, wherein each primer comprises at least 15 contiguous deoxythymidines.
 6. The method of claim 1, wherein each primer comprises 2, 3, 4, or 5 non-deoxythymidine nucleotides at one end.
 7. The method of claim 6, wherein the non-deoxythymidine nucleotides is selected from the group consisting of 3'-VV, 3'-VTV, 3'-VTVV, 3'-VTVVV, 3'-VTVVTV, 3'-VTTV, 3'-VTTTV, 3'-VTTTVV, and 3'-VVVVVV and combinations thereof, wherein V is deoxyadenosine, deoxycytidine, or deoxyguanosine.

8. The method of claim 1, wherein the mixture comprises about 10-25 % of a primer having a 3'-VV, about 0.5-10 % of a primer having a 3'-VTV, about 0.1-5 % of a primer having a 3'-VTTV, about 0.001-0.5% of a primer having a 3'-VTTTV, and upto about 95 % of a primer having a 3'-VVVVV, wherein V is deoxyadenosine, deoxycytidine, or deoxyguanosine.

9. The method of claim 8, wherein the mixture comprises about 15-20 % of a primer having a 3'-VV, about 3-6 % of a primer having a 3'-VTV, about 0.5-3 % of a primer having a 3'-VTTV, about 0.005-0.05% of a primer having a 3'-VTTTV, and about 60-80 % of a primer having a 3'-VVVVV, wherein V is deoxyadenosine, deoxycytidine, or deoxyguanosine.

10. A method for obtaining a DNA complementary to a mRNA, the method comprising:
contacting the mRNA having a polyA tail with a primer mixture comprising a plurality of primers wherein each primer comprises at least 10 contiguous deoxythymidines and a non-polyA-complementary region near one end, wherein the non-polyA-complementary region is selected from the group consisting of 3'-VV, 3'-VTV, 3'-VTVV, 3'-VTVVV, 3'-VTVVTV, 3'-VTTV, 3'-VTTTV, 3'-VTVT, and 3'-VVVVV, and combinations thereof, wherein V is deoxyadenosine, deoxycytidine, or deoxyguanosine; and
reverse transcribing the mRNA using a reverse transcriptase to produce a DNA strand complementary to the mRNA.

11. A method of producing uni-directionally cloned complimentary DNA libraries from mRNA, the method comprising:

contacting the mRNA having polyadenylated tails with a primer mixture, wherein each primer in the mixture has at least 10 contiguous deoxythymidines and at least two non-deoxythymidine nucleotides near one end and a double stranded restriction enzyme sequence at the opposite end;

reverse transcribing the mRNA using a reverse transcriptase to produce a DNA strand complementary to the mRNA;

modifying the complementary DNA strand wherein the polyT tail is substantially removed; and

amplifying the modified cDNA strand by inserting the strand into a cloning vector uni-directionally, and amplifying using a DNA polymerase.

12. The method of claim 11, wherein the primer comprises at least 15 contiguous deoxythymidines.

13. The method of claim 11, wherein the primer comprises 2, 3, 4, or 5 non-deoxythymidine nucleotides at one end, wherein not more than 2 non-deoxythymidine nucleotides are contiguous.

14. The method of claim 11, wherein the non-deoxythymidine nucleotides is selected from the group consisting of 3'-VV, 3'-VTV, 3'-VTVV, 3'-VTVVV, 3'-VTVVTV, 3'-VTTV, 3'-VTTTV, 3'-VTTTVV, and 3'-VVVVV and combinations thereof, wherein V is deoxyadenosine, deoxycytidine, or deoxyguanosine.

15. The method of claim 11, wherein the mixture comprises about 10-25 % of a primer having a 3'-VV, about 0.5-10 % of a primer having a 3'-VTV, about 0.1-5 % of a primer having a 3'-VTTTV, about 0.001-0.5% of a primer having a 3'-VTTTVV, and upto about 95 % of a primer having a 3'-VVVVV, wherein V is deoxyadenosine, deoxycytidine, or deoxyguanosine.

16. The method of claim 15, wherein the mixture comprises about 15-20 % of a primer having a 3'-VV, about 3-6 % of a primer having a 3'-VTV, about 0.5-3 % of a primer having a 3'-VTTTV, about 0.005-0.05% of a primer having a 3'-VTTTVV, and about 60-80 % of a primer having a 3'-VVVVV, wherein V is deoxyadenosine, deoxycytidine, or deoxyguanosine.

17. A method of producing uni-directionally cloned complimentary DNA libraries from mRNA, the method comprising:

contacting the mRNA having a polyA tail with a primer mixture wherein each primer in the mixture has at least 15 contiguous deoxythymidines having a restriction enzyme site at one end and a non-polyA-complementary region near the opposite end, wherein the non-polyA-complementary region is selected from the group consisting of 3'-VV, 3'-VTV, 3'-VTVV, 3'-VTVVV, 3'-VTVVTV, 3'-VTTV, 3'-VTTTV, 3'-VVTVVV, and 3'-VVVVV, and combinations thereof, wherein V is deoxyadenosine, deoxycytidine, or deoxyguanosine;

reverse transcribing the mRNA using a reverse transcriptase to produce a cDNA strand having a polyT tail;

modifying the cDNA strand wherein the polyT tail is substantially removed; and

amplifying the modified cDNA strand by inserting the strand into cloning vector unidirectionally, and amplifying using a DNA polymerase.

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